

REVIEW

Cannabinoid CB₂ receptors in human brain inflammation

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The presence of functional cannabinoid CB₂ receptors in the CNS has provoked considerable controversy over the past few years. Formerly considered as an exclusively peripheral receptor, it is now accepted that it is also present in limited amounts and distinct locations in the brain of several animal species, including humans. Furthermore, the inducible nature of these receptors under neuroinflammatory conditions, in contrast to CB₁, makes them attractive targets for the development of novel therapeutic approaches. In fact, the undesired psychoactive effects caused by CB₁ activation have largely limited the clinical use of cannabinoid-related compounds that act on these receptors. In this review some recent findings on the anti-inflammatory properties of CB₂ receptors are presented, as well as new perspectives that have been obtained based on studies of human postmortem brain samples. In addition, various working hypotheses are also proposed and discussed. *British Journal of Pharmacology* (2008) **153**, 277–285; doi:10.1038/sj.bjp.0707505; published online 15 October 2007

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Abbreviations: A β , β -amyloid peptide; AD, Alzheimer's disease; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; IL-1, interleukin-1; MS, multiple sclerosis; THC, Δ -9-tetrahydrocannabinol

CB₂ receptors in neuroinflammation

Data obtained during the past few years have shown that natural and synthetic cannabinoids are neuroprotective after various types of insults (reviewed by Fernández-Ruiz *et al.*, 2005). These beneficial effects were thought to be mediated mainly by cannabinoid receptors of the CB₁ type, as this receptor is expressed at a high level in the CNS and its activation triggers several mechanisms that protect neurons from death (Howlett *et al.*, 2002). Included in these are inhibition of cell excitability and a decrease in the release of glutamate and other neurotransmitters. Cannabinoids act on glia and neurons to inhibit the release of proinflammatory molecules, including interleukin-1 (IL-1), tumour necrosis factor- α (TNF- α), IL-6 and nitric oxide (Molina-Holgado *et al.*, 1997, 1998, 2002b; Shohami *et al.*, 1997; Puffenbarger *et al.*, 2000; Cabral *et al.*, 2001), and enhance the release of the anti-inflammatory cytokines IL-4, IL-10 (Klein *et al.*, 2000) and an IL-1 receptor antagonist (Molina-Holgado *et al.*, 2003). Due to the neuronal and, specifically, presynaptic location of CB₁ receptors, these actions were thought to be exerted directly on local neural circuits. More recently, however, non-CB₁-mediated protective effects of cannabinoids have also been reported (such as antioxidative actions

and *N*-methyl-D-aspartic acid-antagonism; reviewed by Fernández-Ruiz *et al.*, 2005) thus prompting the search for additional mechanisms. In addition, other elements of the endocannabinoid system (ECS) such as the enzymes involved in endocannabinoid degradation or the yet to be characterized uptake carrier have been considered as putative pharmacological targets.

Interestingly, growing attention is being paid to the second cannabinoid receptor, CB₂. Initial studies revealed that this receptor was expressed exclusively in peripheral tissues. Specifically, CB₂ receptors have been demonstrated in cells and tissues of the immune system, such as the marginal zone of the spleen (Lynn and Herkenham, 1994). In contrast to the constitutive presence of CB₁, recent studies have confirmed the inducible nature of this receptor in other tissues and organs including the CNS, although CB₁ receptors may also be upregulated under pathological conditions, such as, for instance, ischaemia (Jin *et al.*, 2000; Fernández-López *et al.*, 2006). This latter finding is supported by results obtained by Cabral and co-workers, from observing the pattern of expression of both receptors during microglia differentiation using an *in vitro* model of multistep activation (Carlisle *et al.*, 2002; Cabral and Marciano-Cabral, 2005). Additional studies have confirmed a key role for CB₂ in macrophage/microglia functions (Table 1). At present, it is known that the anti-inflammatory properties of cannabinoid agonists also involve CB₂

Table 1 CB₂ receptor and microglial function (*in vitro* data)

Reference	Insult/ challenge	Ligand(s)	Receptor mediation	Effects of cannabinoid agonists	Signalling pathways involved	Cell type
Carlisle <i>et al.</i> (2002)	Thioglycolate LPS IFN- γ	None	CB ₂ mediated	\uparrow CB ₂ expression with cell activation	Not studied	Murine and rat peritoneal macrophages Murine RAW264.7 Murine P388D1 Rat microglia BV-2 Mouse microglia
Walter <i>et al.</i> (2003)	ATP	AEA 2-AG PEA SR141716A SR144528 Cannabinol Cannabidiol O-1918	CB ₂ mediated abn-CBD mediated	\uparrow Cell migration	ERK1/2	BV-2 Mouse microglia
Klegeris <i>et al.</i> (2003)	LPS + IFN- γ	JWH-015 SR141716A SR144528	CB ₂ mediated	\downarrow IL-1 β \downarrow TNF- α	Not studied	THP-1 Human microglia
Franklin and Stella (2003)	None	ACPA Cannabinol Cannabidiol O-1918 SR141716A SR144528	CB ₂ mediated abn-CBD mediated	\uparrow Cell migration	Gi/Go	BV-2
Carrier <i>et al.</i> (2004)	M-CSF	2-AG AEA JWH133 SR144528	CB ₂ mediated	\uparrow Proliferation	ERK1	RTMGL1
Ramírez <i>et al.</i> (2005)	A β 25-35 and A β 1-40	HU-210 WIN55212-2 JWH-133	CB ₁ mediated CB ₂ mediated	\downarrow Morphological changes \downarrow MTT \downarrow TNF- α \uparrow Neuronal survival \downarrow NO \downarrow iNOS \downarrow Cytokines (IL-1 β , IL-6, TNF- α)	Not studied	Mouse microglia
Ortega-Gutierrez <i>et al.</i> (2005a)	LPS	UCM707 OMDM1 AEA Methanandamide SR141716A SR144528	CB ₁ mediated CB ₂ mediated	\downarrow IFN- γ -mediated CD40 expression \downarrow TNF- α \downarrow NO \uparrow Phagocytosis \uparrow CB ₂	Not studied	Mouse microglia
Ehrhart <i>et al.</i> (2005)	A β 1-42	JWH-015	CB ₂ mediated	\downarrow IFN- γ -mediated CD40 expression \downarrow TNF- α \downarrow NO \uparrow Phagocytosis \uparrow CB ₂	JAK/STAT1	Mouse microglia
Maresz <i>et al.</i> (2005)	GM-CSF IFN- γ LPS	None	Not studied	\uparrow CB ₂	Not studied	Mouse microglia
Eljaschewitsch <i>et al.</i> (2006)	NMDA OGD	AEA WIN55212-2 AM251 AM630	CB ₁ mediated CB ₂ mediated	\uparrow MKP-1 \downarrow NO \downarrow iNOS	ERK-1/2	Rat microglia BV-2 OHSCs
Mukhopadhyay <i>et al.</i> (2006)	LPS	None	Not studied	\uparrow CB ₂	NF- κ B PKA PKC	RAW264.7
Kreutz <i>et al.</i> (2007)	NMDA	THC AEA 2-AG AM630	CB ₂ mediated (THC, 2-AG) Non-CB ₂ mediated (AEA)	\downarrow Number of microglial cells \downarrow Number of degenerating neurons (2-AG)	Not studied	OHSCs

receptors. CB₂ receptor activation decreases the *in vitro* production of proinflammatory molecules in a number of neural cell types, such as rat microglial cells (Puffenbarger *et al.*, 2000; Facchinetti *et al.*, 2003), primary mouse astrocytes (Molina-Holgado *et al.*, 1997, 2002a), human microglial and THP-1 cells (Klegeris *et al.*, 2003), and human astrocytes (Sheng *et al.*, 2005). The activation of CB₂ receptors also

reduces the release of proinflammatory factors in animal models of perinatal hypoxia-ischaemia (Fernández-López *et al.*, 2006) and Huntington's disease (Fernández-Ruiz *et al.*, 2005).

Most of these data have been obtained in primary microglial cultures or with murine cell lines *in vitro*. As pointed out by Maresz *et al.* (2005), the upregulation of

Table 2 CB₂ receptors and microglial function (*in vivo* data)

Reference	Insult/disease model	Ligand(s)	Receptor mediation	Molecular effects of cannabinoid agonists	Symptomatic effects of cannabinoid agonists	Animal species
Arevalo-Martin <i>et al.</i> (2003)	Theiler's virus	WIN55212-2 ACEA JWH-015	CB ₁ mediated CB ₂ mediated	↓ Microglial activation ↓ MHC-II expression ↓ CD4 ⁺ infiltration	Motor recovery Remyelination	Mouse
Zhang <i>et al.</i> (2003)	Chronic constriction injury Freund's complete adjuvant injection Spinal nerve ligation	None	↑ CB ₂ mRNA	Not studied	Not studied	Rat
Maresz <i>et al.</i> (2005)	Experimental autoimmune encephalomyelitis	None	Not studied	↑ CB ₂	Not studied	Mouse
Beltramo <i>et al.</i> (2006)	Spinal cord ligation	AM1241 L768242 SR144528	CB ₂ mediated	↑ CB ₂	↓ Hyperalgesia	Mouse
Mukhopadhyay <i>et al.</i> (2006)	LPS	None	Not studied	↑ CB ₂	Not studied	Rat
Ashton <i>et al.</i> (2007)	Middle cerebral artery occlusion Hypoxia-ischemia	None	Not studied	↑ CB ₂	Not studied	Rat

Table 3 CB₂ receptors in neurodegenerative diseases

Control	Alzheimer's disease	Down's syndrome	SIVE	HIVE	Multiple sclerosis
Perivascular microglia	Activated microglia	Activated microglia	Activated microglia Perivascular microglia T-lymphocytes	Activated microglia Perivascular microglia T-lymphocytes	Activated microglia Macrophages T-lymphocytes Astrocytes

Abbreviations: HIVE, type 1-human immunodeficiency virus-induced encephalitis; SIVE, Simian immunodeficiency virus-induced encephalitis.

cannabinoid CB₂ receptors also takes place *in vivo*, and seems to be triggered by chronic inflammatory conditions (Table 2). These authors were the first to show that the increased expression of these receptors was a direct consequence of microglial cell activation occurring during an experimentally induced autoimmune process. More recently, additional studies have further corroborated the *in vivo* link between chronic neuroinflammation and CB₂ upregulation in animal models of pain (Beltramo *et al.*, 2006), inflammation (Mukhopadhyay *et al.*, 2006) and ischaemia-induced hypoxia (Ashton *et al.*, 2007). From these studies, it can be concluded that macrophage/microglia activation, whether by inherent changes to *in vitro* conditions or by experimentally induced neuroinflammatory processes, leads to a dramatic increase in CB₂ expression. It should be noted that this glial expression also affects other elements of the ECS, such as, for example, fatty acid amide hydrolase (FAAH). Albeit the expression of FAAH in microglia is negligible (Stella, 2004), it seems to play a significant role in astrocytic function.

Few data exist on the role that CB₂ receptors may play in humans. Due to its abundant presence in immune-related cells, it seems reasonable to think that they are involved in the well-known effects of cannabinoids on immunological function (Klein, 2005). As discussed in subsequent sections of this review, in the human CNS, CB₂ receptors seem to follow a similar pattern of inducible expression as that described in animal models.

CB₂ receptors in neuroinflammatory conditions of the human brain

Although inflammation serves as a protective function in controlling infections and promoting tissue repair, it can also cause tissue damage and disease. Recently, 'neuroinflammation' became a commonly used term and neuroscientists spoke of 'reactive gliosis' when describing endogenous CNS tissue responses to injury. Neuroinflammation incorporates a wide spectrum of complex cellular responses that include activation of microglia and astrocytes and elaboration of cytokines and chemokines, complement proteins, acute phase proteins and related molecular processes. In addition, invasion of peripheral immune cells is also usually present. These events may have detrimental effects on neuronal function, leading to neuronal injury with further glial activation and, ultimately, neurodegeneration. Neuroinflammation occurs in myelin degenerative disorders, such as multiple sclerosis (MS, reviewed by Martino *et al.*, 2002) and also in neurodegenerative disorders, such as Alzheimer's disease (AD, McGeer and Rogers, 1992) Parkinson's disease (McGeer *et al.*, 2001) and Huntington's disease (Sapp *et al.*, 2001), viral encephalitis (Gendelman *et al.*, 1994), ischaemia (Chopp *et al.*, 1994) and traumatic brain injury (Dusart and Schwab, 1994). The release of proinflammatory and neurotoxic mediators (interferon- γ , tumour necrosis factor- α , IL-1 β , IL-6, eicosanoids, nitric oxide and reactive oxygen species) may induce or aggravate

brain damage. These factors are produced by glial cells and invading immune cells (mainly reactive microglia) and can be deleterious to neurons (for review see Boje and Arora, 1992; Chao *et al.*, 1992; Mc Guire *et al.*, 2001; Liu and Hong, 2003).

On the other hand, the study of the changes in the expression pattern of several elements of the ECS in the healthy versus diseased human brain has provided new perspectives to the field (Pazos *et al.*, 2005; Benito *et al.*, 2007c). Specifically, recent evidence suggests that the ECS may participate in the pathogenesis and/or the adaptive changes taking place in the human CNS after chronic neuroinflammatory conditions. As previously mentioned, this participation would include, in addition to the known neuroprotection exerted by neuronal CB₁ receptors, glial CB₂ receptors and FAAH. Both proteins seem to be significantly upregulated in microglial and astroglial cells, respectively, in areas of active neuroinflammation. Among these, β -amyloid enriched neuritic plaques in AD, infiltrative areas in viral encephalitis and regions of active demyelination in MS show marked increases in CB₂ and FAAH levels of glial expression (reviewed by Benito *et al.*, 2007c).

Alzheimer's disease

AD accounts for the most frequent form of dementia in the elderly and is one of the most important health challenges in western countries. In 2001, more than 5 million people with dementia lived in European countries and more than 4 million people were affected in the United States alone. Furthermore, a 100% increase in the number affected is expected in developed regions by the year 2040 (Ferri *et al.*, 2005). The initial symptoms presented in AD patients usually include slight losses of memory and progress to a total inability to control basic functions of the body. Current treatments for AD only provide symptomatic relief and much effort is being directed to the search for curative and/or preventive treatments (Lleó *et al.*, 2006).

Although the neuropathological and molecular changes that underlie these symptoms are now well characterized thanks to the analysis of postmortem samples, controversial aspects still await further clarification. In general terms, the 'amyloid hypothesis' is now accepted (Walsh and Selkoe, 2004). According to this hypothesis, the aberrant processing of a peptide located on the cell membrane leads to the synthesis of a small 42 amino-acid peptide (the amyloid peptide, A β) that exhibits a remarkable tendency to acquire a tridimensional structure that makes it precipitate in the extracellular space. This is the origin of the 'amyloid or neuritic plaques' that accumulate in the brain parenchyma of AD patients and that are thought to trigger a cascade of events that leads to massive neuronal death (Giulian, 1999). Among these events, amyloid deposition (i) stimulates the hyperphosphorylation of the cytoskeletal tau protein, thus triggering its precipitation and leading to the formation of intraneuronal 'neurofibrillary tangles'; (ii) triggers a potent, local inflammatory reaction, involving microglial and astroglial cells that attempt to encapsulate and degrade the amyloid deposit; (iii) results in a massive local accumulation of inflammatory cytokines and reactive oxygen species

('cytokine cycle'; Mrak and Griffin, 2005) and (iv) leads ultimately to neuronal death and massive loss of functional synapses, thus dramatically altering neurotransmission (Wyss-Coray and Mucke, 2002).

Therapeutic approaches intended to decrease endogenous A β levels are considered to be among the most promising strategies for the treatment of AD. Clinical trials with vaccines against this peptide have been carried out, although the appearance of meningoencephalitis in approximately 6% of patients has led to this treatment being prematurely stopped (Goni and Sigurdsson, 2005). The possible application of anti-inflammatory compounds is also under debate; in particular, non-steroidal anti-inflammatory drugs, cyclooxygenase-2 inhibitors and peroxisome-proliferator-activated receptor ligands have been tested (reviewed by Aisen, 2002).

As mentioned above, undesired psychoactive effects have limited the clinical use of cannabinoid-related chemicals. Memory impairment is one of the most frequent side effects of these compounds, so their usefulness for the treatment of AD has been seriously questioned. However, a pilot study by Volicer *et al.* (1997) performed in patients with dementia who were refusing to eat indicated that dronabinol (Δ -9-tetrahydrocannabinol, THC) significantly improved this condition and the behaviour of these patients, with few side effects. From these results, the investigators proposed that dronabinol could be a suitable compound for the treatment of anorexia and disturbed behaviour in AD patients. Further, in accordance with this proposal, Walther *et al.* (2006) found that dronabinol also improved nocturnal agitation and motor activity in six patients in the late stages of dementia. However, more research is needed to substantiate the effectiveness of this compound for the treatment of AD.

Several studies performed *in vitro* and in animal models of AD have shown a protective role for cannabinoids. The molecular basis of these effects includes CB₁-, CB₂- and also receptor-independent mechanisms (for a recent review, see Benito *et al.*, 2007a).

The analysis of human post-mortem brain samples from AD patients has provided information on the neuropathology of the ECS and has allowed new hypotheses to be formulated on the possible role of this system in the prevention and/or treatment of AD. Data from AD patients may be summarized as follows: (i) in AD brains, CB₁ receptor binding in hippocampus and basal ganglia structures is decreased (Westlake *et al.*, 1994), but not in neocortex or frontal cortex and the receptor is less efficiently coupled to signal transduction mechanisms (Ramírez *et al.*, 2005); (ii) A β deposition induces dramatic changes in the phenotype of glial cells, including upregulation of some elements of the ECS (such as CB₂ receptors and FAAH) (Benito *et al.*, 2003); (iii) overexpression of CB₂ and FAAH seems to be a phenomenon directly linked to A β deposition, as suggested by the study of human samples of Down's syndrome, a natural model of AD (Núñez *et al.*, submitted) and, (iv) the upregulation of these elements of the ECS is a cell-specific event, as CB₂ receptors seem to be restricted to microglia and FAAH to astrocytes. Although the functions of these receptors in the CNS are far from clear, they may be now considered as diagnostic markers for microglial activation

and as relevant candidates for the development of anti-A β therapies. Indeed, in the next section of the review, we will cover the *in vitro* data illustrating the anti-inflammatory effects of CB₂ agonists.

More recently, we have explored a possible functional role for microglial CB₂ receptors in A β removal (Núñez *et al.*, unpublished observations). It is known that one of the most important functions of microglial cells in AD is the removal of pathological proteinaceous deposits, such as neuritic plaques. Wyss-Coray *et al.* (2003) showed that adult mouse astrocytes may also participate in this process, suggesting a new way for therapeutic intervention. Our results indicate that the CB₂ agonist JWH-015 induces the removal of A β plaques from human AD tissue sections by human THP-1-derived macrophages, but not by other types of glioma cell lines. Furthermore, this effect was achieved at low concentrations (maximum effect at 5 nM) and was reversed by the CB₂ selective antagonist SR144528. Interestingly, Ehrhart *et al.* (2005) have recently shown that this same compound enhances A β phagocytosis by attenuating CD40-mediated inhibition in microglial cells. In light of these observations, we speculate that this effect could be related to the CB₂-mediated inhibition of the release of IL-1 and tumour necrosis factor- α by stimulated THP-1 cells (Klegeris *et al.*, 2003). As shown by Koenigsnecht-Talboo and Landreth (2005), a pro-inflammatory milieu results in a marked interference with the ability of microglia to phagocytose A β . Therefore, CB₂ receptor activation might afford an additional advantage, as it could also decrease local neuroinflammation thus enhancing A β removal *in situ*.

In summary, CB₂ activation could provide beneficial effects in AD through several mechanisms, including a decrease in local, microglia-mediated inflammation and an enhancement of A β removal.

HIV-induced encephalitis

As mentioned before, the presence of CB₂ receptors in the healthy human brain is rather limited. In fact, we have only detected significant levels of CB₂ immunoreactivity in a discrete population of perivascular cells (Núñez *et al.*, 2004). These cells were identified as perivascular macrophages. Although considered a part of the blood brain barrier, these cells of myeloid origin exhibit distinct properties from other cell types in the CNS (for a comprehensive review, see Williams and Hickey, 2002). Their selective location embracing the external wall of the blood vessels places them in a privileged position to participate in the control of the entry of exogenous elements into the CNS. In addition, they are considered transient CNS residents, as they are continuously replaced by bone marrow-derived monocytes.

It was this selective CB₂ receptor expression that turned our attention to the study of their possible relevance in the encephalitis induced by the type 1-human immunodeficiency virus. This inflammatory process underlies the clinical paradigm of acquired immunodeficiency syndrome dementia that affects at least 5–7% of acquired immunodeficiency syndrome patients per year, in Europe alone (Mollace *et al.*, 2001). These patients exhibit a myriad of cognitive and motor symptoms, including leg weakness,

memory loss, apathy, social withdrawal and personality changes. In its more advanced and severe form, the disease ultimately leads the patient progressing to a vegetative state (González-Scarano and Martín-García, 2005). Interestingly, children's brains seem to be more vulnerable, probably due to the immaturity of the blood brain barrier when they get infected.

It is currently thought that the entry of the HIV-1 into the CNS follows a 'Trojan horse' strategy (Kaul *et al.*, 2001). According to this line of reasoning, peripheral infected monocytes committed to replace perivascular macrophages act as carriers of the virus. Thus, all the virus found in the brain is probably initially derived from monocytes that have differentiated into perivascular macrophages (González-Scarano and Martín-García, 2005). Once inside the CNS, productively infected macrophages constitute a source of virus that acts on microglia. Neurons themselves seem not to be directly infected. Thus, both pericytes and parenchymal microglia are the two main cell types involved in this process that leads to the formation of the so-called 'multinucleated giant cells'. Finally, several mechanisms (including oxidative stress, production and release of pro-inflammatory cytokines, and/or direct injury by viral proteins) lead to neurodegeneration and subsequent clinical symptoms (González-Scarano and Martín-García, 2005).

As with other types of G-protein-coupled receptors, the CB₂ is upregulated in perivascular macrophages as a consequence of the HIV-1-triggered inflammatory process. Specifically, chemokine receptors of the CCR3 and CCR5 type have been reported to be upregulated in Simian immunodeficiency virus-induced encephalitis and type 1-human immunodeficiency virus-induced encephalitis brains (Cartier *et al.*, 2005). Using samples from macaque and human infected brains, we observed that only those samples from infected individuals with encephalitis showed high levels of CB₂ expression, in contrast with those from controls and infected individuals without encephalitis. The increases in CB₂ expression were especially evident in perivascular macrophages and microglial cuffs (Benito *et al.*, 2005).

Interestingly, infiltrated T lymphocytes also show strong immunoreactivity for CB₂ receptors. The entry of these cells from the periphery into the CNS is an additional feature of type 1-human immunodeficiency virus-induced encephalitis, although their contribution to the virus pool in the CNS is not clear, mainly because genotypic and phenotypic analyses show that viruses from the brain are more similar to those from monocytes and macrophages than to those from T lymphocytes (González-Scarano and Martín-García, 2005). Ghosh *et al.* (2006) have recently shown that CB₂ activation inhibits the transendothelial migration of Jurkat T cells and human primary T lymphocytes by interfering with the CXCL12/CXCR4 system. This interaction seems to take place by cross-talk between their signal transduction routes. Very recently, a similar downstream interaction has been described for the CCR5 receptor (GA Cabral *et al.*, personal communication).

Thus, CB₂ receptors might participate in the inflammatory response against viral infection of the brain by modifying the microglial production of inflammatory molecules and by modulating the entry of peripheral cells into the CNS.

Multiple sclerosis

MS is the major cause of neurological disability among young adults in North America and Europe (Noseworthy *et al.*, 2000). Its aetiology is unknown but much evidence suggests that genetic and environmental factors may have an important role on MS susceptibility, although the possibility of a role for infectious agents has also been considered (Frohman *et al.*, 2006). The neuropathology of this inflammatory and demyelinating disease of the CNS includes axonal degeneration, oligodendrocyte loss and subsequent induction of well-demarcated hypocellular and demyelinated areas (Frohman *et al.*, 2006). Lymphocytes and monocytes infiltrate the white matter surrounding the blood vessels, destroying myelin, while axons are not directly damaged. Its characteristic symptoms (such as painful muscle spasms, tremor, ataxia, weakness or paralysis) are thought to be the result of new lesions and expansion of old lesions at the CNS level as a result of myelin phagocytic activity carried out by cells of monocytic origin (for a review, see Noseworthy *et al.*, 2000).

The ECS constitutes a promising target for the development of new drugs for the treatment of MS (Pryce and Baker, 2005). Interest in the potential of cannabinoids as a treatment for the symptoms of MS is evidenced by results obtained in clinical trials performed during the last few years. A randomized, placebo-controlled trial in which stable MS and muscle spasticity patients were treated with cannabis extract or Δ^9 -THC for 15 weeks showed that cannabinoids did not exhibit a beneficial effect on spasticity, as assessed with the Ashworth scale (Zajicek *et al.*, 2003). However, these patients reported an improvement in pain relief as well as in mobility. In addition, patients who continued Δ^9 -THC treatment for up to 12 months showed a small reduction of spasticity (Zajicek *et al.*, 2005). The oromucosal spray Sativex (which contains Δ^9 -THC and cannabidiol) tested in another clinical trial showed a significant reduction in pain and sleep disturbances in patients with central neuropathic pain syndromes due to MS (Rog *et al.*, 2005).

These data are supported by results obtained in different animal models of MS. Thus, THC administration delayed the onset of the disease and markedly reduced CNS inflammation (Lyman *et al.*, 1989) and synthetic cannabinoids ameliorated tremor and spasticity through a CB₁-mediated mechanism (Baker *et al.*, 2000). In addition, they improved neurological deficits as a result of parallel reduction in CNS inflammation and extensive remyelination (Arevalo-Martin *et al.*, 2003) and produced beneficial effects on motor activity, accompanied by a reduction of damage to axons, through CB₁ and CB₂ receptor activation (Docagne *et al.*, 2007).

So far, the treatment of MS has focused on CB₁ activation. However, results from animals models (Arevalo-Martin *et al.*, 2003; Docagne *et al.*, 2007) and in human samples (Yiangou *et al.*, 2006; Benito *et al.*, 2007b) suggest that CB₂ receptors are potential therapeutic targets for the treatment of MS. Moreover, the probability of finding cannabinoid-based drugs devoid of undesirable psychotropic side effects for treating this disease now seems more likely.

The studies performed by Yiangou *et al.* (2006) and Benito *et al.* (2007b) in human spinal cord and brain MS samples,

respectively, indicated the presence of strong CB₂-immunoreactivity in microglia/macrophages in areas of white matter, usually within active plaques or in the periphery of chronic lesions. These results confirm that CB₂ expression in glial cells in the human CNS is upregulated, as previously found in other neuroinflammatory conditions (Benito *et al.*, 2003, 2005) and even in healthy human brains (Núñez *et al.*, 2004). Importantly, we showed that a fraction of the CB₂-positive macrophages also contained myelin basic protein, indicating recent phagocytic activity and suggesting that CB₂ receptor expression in plaque-associated macrophages may be an early event in plaque evolution. Several *in vitro* studies have documented that microglia/macrophages are involved in phagocytosis of myelin debris in MS lesions and that this process triggers release of pro-inflammatory cytokines and nitric oxide (Williams *et al.*, 1994; Mosley and Cuzner, 1996; van der Laan *et al.*, 1996). Although little is known of the effects of cannabinoids on myelin phagocytosis, previous studies have shown that the activation of the ECS decreases the production of pro-inflammatory cytokines and levels of nitric oxide in macrophages/microglia. This process could account for the anti-inflammatory effect that seems to potentiate neuroprotection induced by cannabinoids (Mestre *et al.*, 2005; Ortega-Gutierrez *et al.*, 2005b). In addition, several characteristics of macrophages such as migration, presentation of peptide antigens or phagocytosis of foreign particles are also significantly influenced by cannabinoids (reviewed by Croxford and Yamamura, 2005).

Our immunohistochemical study performed on human tissue sections shows highest levels of CB₂ receptor immunoreactivity in microglia (Benito *et al.*, 2007b). Further, the distribution of these CB₂-positive cells correlated with that of major histocompatibility complex type II-positive cells. The similarity between CB₂-positive cells and microglia and the colocalization of CB₂-receptors with D-region related human leukocyte-associated antigen led us to suggest the CB₂ receptor as a marker for the identification of MS plaques as they may indicate the evolution grade of demyelination areas. These major histocompatibility complex type II-positive cells are used as defining markers of plaque subtype depending on their localization and abundance in MS lesions (Trapp *et al.*, 1999). Thus, major histocompatibility complex type II-positive cells are abundant throughout the entire extension of acutely active plaques, but are restricted to the periphery of chronic ones.

We also demonstrated CB₂ receptor expression in perivascular T lymphocytes. The myelin-reactive T lymphocytes are thought to be involved in the demyelinating process and cause inflammation (Frohman *et al.*, 2006). It is important to note that cannabinoids decrease CD4⁺ infiltration into the spinal cord in an animal model of MS through CB₁ and CB₂ activation (Arevalo-Martin *et al.*, 2003). Thus, the presence of cannabinoid receptors in T lymphocytes is suggestive of a possible role of the ECS in MS-linked, T-cell-mediated neuroinflammation.

Surprisingly, the CB₂ receptor has also been found to be expressed in white matter astrocytes (Benito *et al.*, 2007b), while not to being expressed by astrocytes in other pathologies such as AD (Benito *et al.*, 2003; Ramírez *et al.*, 2005), type 1-human immunodeficiency virus-induced

encephalitis or Simian immunodeficiency virus-induced encephalitis (Benito *et al.*, 2005). There are few data about the role of CB₂ receptors in astrocytes, although studies *in vitro* suggest that they may modulate the production of different inflammatory mediators (Ortega-Gutierrez *et al.*, 2005a,b; Sheng *et al.*, 2005). More recently, Docagne *et al.* (2007) proposed a neuroprotective effect in an MS animal model as a result of the concomitant activation of CB₁ receptors in neurons and CB₂ in astrocytes.

In summary, cannabinoids are known to have a therapeutic effect in MS. New evidence suggests that the CB₂ receptor could also be a pharmacological target, as its expression is increased in several cell types known to be directly involved in the pathogenesis of MS. The activity of microglia, astrocytes and infiltrated lymphocytes could be modified by the activation of CB₂ receptors.

Conclusions

CB₂ receptors have been found to be present in the CNS of several animal species (Benito *et al.*, 2003; Maresz *et al.*, 2005; Van Sickle *et al.*, 2005), thus offering new opportunities for the pharmacological use of cannabinoid agents. Furthermore, the fact that their expression is increased by inflammatory stimuli suggests that they may be involved in the pathogenesis and/or in the endogenous response to injury. Data obtained *in vitro* and in animal models show that CB₂ receptors may be part of the general neuroprotective action of the ECS by decreasing glial reactivity. Neuropathological findings in human brains (summarized in Table 3) suggest that the upregulation of CB₂ receptors is a common pattern of response against different types of chronic injury of the human CNS. In addition, their selective presence in microglial cells is highly suggestive of an important role in disease-associated neuroinflammatory processes. The anti-inflammatory effects triggered by the activation of the CB₂ receptor make it an attractive target for the development of novel anti-inflammatory therapies. In any case, further research is needed to corroborate the potential usefulness of cannabinoid-based treatments devoid of undesired psychoactive effects.

Conflict of interest

The authors state no conflict of interest.

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